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—Review—
Review Series: Animal Bioresource in Japan

Genetic Materials at the Gene Engineering Division, RIKEN BioResource Center

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Abstract: Genetic materials are one of the most important and fundamental research resources for studying biological phenomena. Scientific need for genetic materials has been increasing and will never cease. Ever since it was established as RIKEN DNA Bank in 1987, the Gene Engineering Division of RIKEN BioResource Center (BRC) has been engaged in the collection, maintenance, storage, propagation, quality control, and distribution of genetic resources developed mainly by the Japanese research community. When RIKEN BRC was inaugurated in 2001, RIKEN DNA Bank was incorporated as one of its six Divisions, the Gene Engineering Division. The Gene Engineering Division was selected as a core facility for the genetic resources of mammalian and microbe origin by the National BioResource Project (NBRP) of the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan in 2002. With support from the scientific community, the Division now holds over 3 million clones of genetic materials for distribution. The genetic resources include cloned DNAs, gene libraries (e.g., cDNA and genomic DNA cloned into phage, cosmid, BAC, phosmid, and YAC), vectors, hosts, recombinant viruses, and ordered library sets derived from animal cells, including human and mouse cells, microorganisms, and viruses. Recently genetic materials produced by a few MEXT national research projects were transferred to the Gene Engineering Division for further dissemination. The Gene Engineering Division performs rigorous quality control of reproducibility, restriction enzyme mapping and nucleotide sequences of clones to ensure the reproducibility of *in vivo* and *in vitro* experiments. Users can easily access our genetic materials through the internet and obtain the DNA resources for a minimal fee. Not only the materials, but also information of features and technology related to the materials are provided via the web site of RIKEN BRC. Training courses are also given to transfer the technology for handling viral vectors. RIKEN BRC supports scientists around the world in the use of valuable genetic materials.
Key words: genetic material, genome, recombinant virus, quality control

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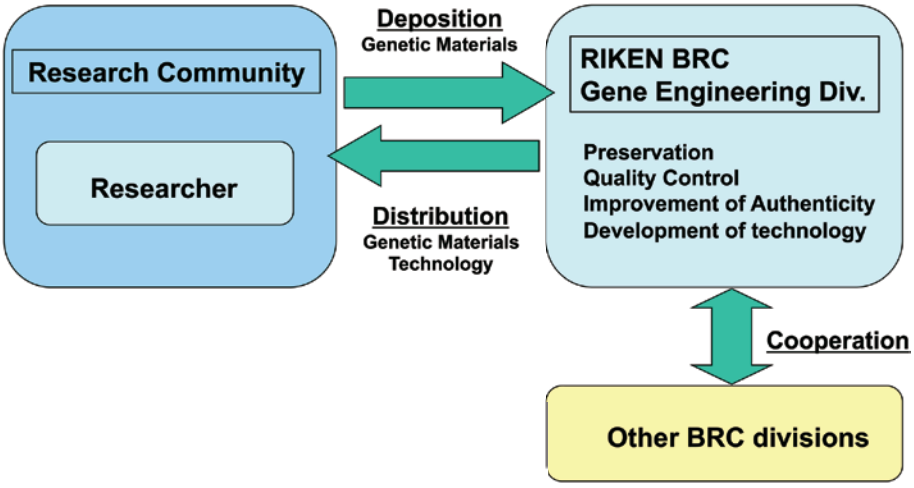

Fig. 1. Schematic representation of banking of genetic materials. RIKEN BRC is engaged in the collection, maintenance, storage, propagation, quality control, and distribution of genetic resources.

Introduction

The RIKEN DNA Bank was organized in June 1987 by the Science and Technology Agency of the Japanese government in accordance with a report by a scientific committee that called for a central repository of genetic resources that would serve scientists in Japan and Asia. In 2001, RIKEN BRC was established within RIKEN and the DNA Bank was merged into RIKEN BRC as the Gene Engineering Division. In 2002, the Gene Engineering Division was selected as a core facility for the collection, preservation and distribution of genetic resources of animal and microbe origin by the MEXT NBRP (Fig. 1). With the support of the scientific community, RIKEN BRC has collected over 3 million excellent materials such as genomic clones and libraries, expressed sequence tag (EST) and cDNA clone sets, expression clone sets, backbone vectors, recombinant adenoviruses and shuttle vectors, and genomic DNA (Fig. 2, Table 1) for the study of human diseases and gene functions. Users can easily access our genetic materials through the internet (Fig. 3) and obtain DNA resources for a minimal fee. Not only the materials, but also information of technology and features related to the materials are provided via our web site. We have focused on the following six groups of genetic resources to promote life sciences: (1) large scaled genome clones from animals and microorganisms; (2) recombinant viruses for gene transfer; (3) DNAs encompassing various promoter-reporter regions; (4) Japanese-specific genetic resources; (5) full-length cDNA sets of growth factors, receptors, transcription factors, replication factors, apoptosis, the cell cycle, signal cascades, and so on; and (6) genetic resources related to the conservation of biodiversity and reference materials for studies of biological classification.

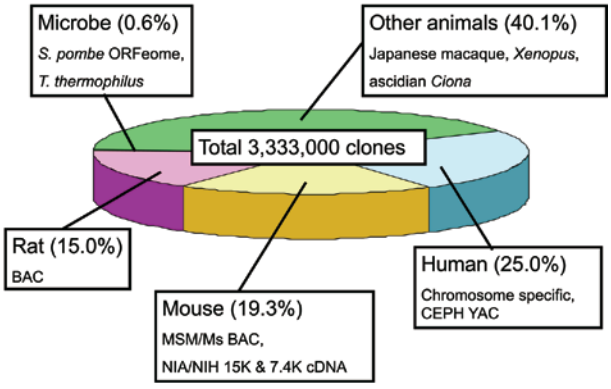

Fig. 2. Collection of genetic materials at the Gene Engineering Division. The numbers in parentheses indicate the relative proportions (%) of each group.

Table 1. Genetic materials available from RIKEN BRC Gene Engineering Division

Group of resources	Name of resources
Genomic clones and library	Human chromosome clone set
	CEPH human mega YAC clone set
	Chimpanzee 22th chromosome clone set
	MSM/Ms mouse BAC clone set
	NBRP mouse C57BL/6N BAC clone set
	NBRP rat BAC clone set
	NBRP Japanese macaque BAC clone set
	Human, mouse, nematoda, and yeast cDNA libraries
EST and cDNA clone set	NIA/NIH mouse cDNA clone set
	Mouse EST clones (ERATO)
	Xenopus laevis and *tropicalis* EST clones (NBRP)
	Ciona intestinalis EST clones (NBRP)
	Genome network project human gateway® entry clone set
Expression clone set	RIKEN DNA bank full CDS expression collection
	RIKEN DNA bank promoter and response element collection
	SEREX cDNA clones and libraries
	Human HLA expression cDNA
	Schizosaccharomyces pombe ORFeome clone set
	NBRP *Xenopus laevis* EST clones
	Thermus thermophilus HB8 expression and disruption plasmids
	Aeropyrum pernix and *Sulfolobus tokodaii* expression plasmids
Backbone vectors	Cloning vectors
	Expression vectors
	Shuttle vectors
Recombinant virus-related materials	Recombinant adenovirus
	Shuttle vector for generating recombinant viruses
Genomic DNA	JCM microorganisms genomic DNA
	Mouse genomic DNA

Genetic Materials Available from RIKEN BRC Gene Engineering Division

cDNA clones

A large amount of EST information is useful for determining the rough temporal expression pattern of genes; thus many EST cloned libraries have been established and analyzed. Individual cDNA clones of EST can be used to analyze expression patterns of genes by whole-mount *in situ* hybridization [19]. The EST clones of an ascidian *Ciona intestinalis* comprise *ca*. 452,000 clones derived from 16 developmental stages [25, 26]. Individual clones as well as expression patterns of genes are searchable using the database provided (web site of '*Ciona intestinalis* EST'). NIA/NIH mouse 15K and 7.4K cDNA clone sets were prepared by Dr Minoru Ko and colleagues [14, 29, 33] as results of large scale analyses of EST clones. These results were used for the construction of commercially available microarrays. The mouse 15K clone set contains about 15,000 'unique' cDNA clones rearrayed from 52,374 ESTs from pre- and per-implantation embryos, E12.5 female gonad/mesonephros, and newborn ovaries. The 7.4K clone set was constructed from about 7,400 cDNA clones with no redundancy within the set and NIA Mouse 15K. Human full-CDS clones that are the products of the Genome Network Project (MEXT) are also available.

There are clone sets which are used for the introduction of genes to cultured cells. The *Xenopus* EST clone set contributed to a study of gene expression in the frog. Of six clone sets of *Xenopus laevis*, two EST clone sets (*X. laevis* anterior neuroectoderm EST clones, RDB 6129; *X. laevis* Keller explans EST clones, RDB 6726) are constructed with an expression vector and they are

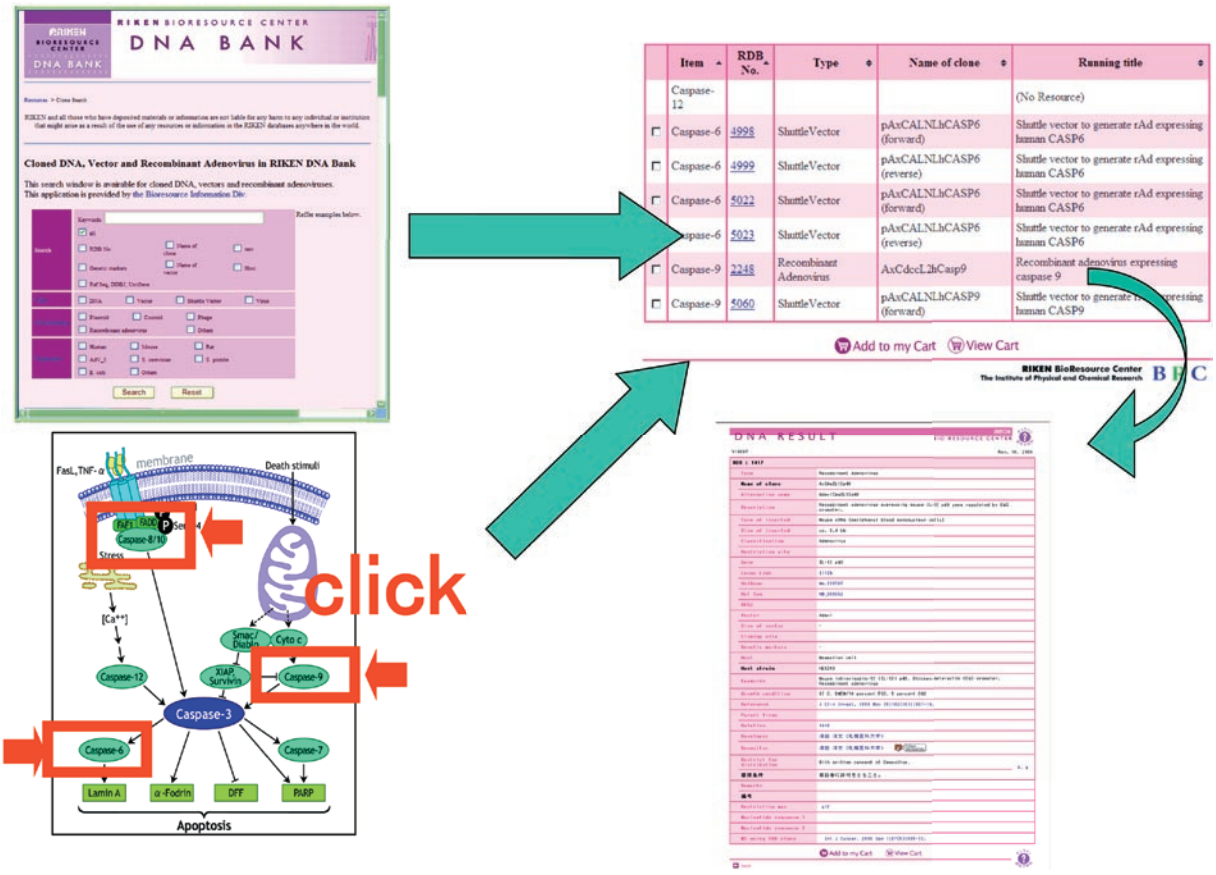

Fig. 3. A scientist can search for materials of interest in the Gene Engineering Division. Information of each resource and the specific terms and conditions regarding distribution of the resource are indicated.

useful for establishing transient expression of genes in the frog oocytes. A human HLA class I cDNA collection isolated from Japanese can be used to express histocompatibility antigen in human cells [2, 3] and is useful for immunology, especially cancer immunology [4, 5, 20]. The human SEREX (serological analysis of cancer antigens by recombinant cDNA expression cloning) clones were initially constructed for the screening of cancer antigens of Japanese patients using a phage expression system [21, 22]. Five hundred plasmid clones were converted from the phages and are available as expression vectors for use in mammalian cells. The Gene Engineering Division also provides full-CDS expression clones that were constructed by our division, and the confirmed nucleotide sequences of each clone and their expression proteins determined by western blotting.

BAC Clones

To compare genetic variations between evolutionally related species and the single nucleotide polymorphism (SNP) among strains of experimental animals, BAC clones have been used for the analysis of genomic DNA of various organisms. The Japanese macaque *Macaca fuscata* BAC (MSB2) consisting of *ca*. 200,000 clones was constructed. Although a complete genome sequence of Japanese macaques is not yet available [11], terminal sequences of the inserted genes have already been analyzed and chromosome mapping of BAC clones are accessible in a database held at the DNA Data Bank of Japan (DDBJ) of the National Institute of Genetics. The MSM/Ms mouse BAC clones have contributed to SNP analysis of Asian mouse subspecies *Mus musculus molossinus* and the C57BL/6J mouse strain by BAC-end sequence analysis [1]. BAC clones from two strains of the rat have also contributed to SNP analysis of strains

[23, 27]. The terminal sequence of the MSM/Ms mouse BAC and two of the rat BAC libraries have already been analyzed and individual clones of the BAC libraries can be easily accessed via the web sites of each BAC library.

The mouse is an important model organism for understanding human diseases. It always plays a key role in functional genomics [34]. Use of the BAC clone is not limited to the investigation of SNP. Recent advances in molecular biology techniques utilize BAC in functional genomic studies of the mouse. BAC clones are used for generation of transgenic mice having additional copies of genes or a genomic region to analyze gene function in the gain-of-function phenotype and in gene rescue for the loss of function mutant and knockout mice. Furthermore, techniques of modification of nucleotide sequences of BAC clones have been developed and applied to the generation of gene modified transgenic mice and gene targeting by substitution of a genomic region with marker genes.

Rats are also used as *in vivo* highly valuable models for hypertension, aging, infectious diseases, cancer, and neurological disorders because of their relevance to human physiology and size advantages in surgical operations over the mouse [10, 27]. However, the lack of efficient tools to manipulate the rat genome and generate transgenic rats has drastically limited the use of this research model. Recent advances in gene expression and transgenic systems have provided new possibilities for the generation of informative rat models [7]. Establishment of rat ES cells [6, 15] and iPS cells [16] may promote the generation of knockout models with BAC clones and will greatly expand the scope of present experimental limitations with new rat resources [27].

Currently, the C57BL/6N (B6N) substrain, in conjunction with B6N embryonic stem (ES) cells, is being used in worldwide knockout mouse projects and it is known as the standard inbred strain with the most uniform genetic background [17]. Although B6N ES cells are available from public resource centers such as the Mutant Mouse Regional Resource Center (MMRRC) at UC Davis and the RIKEN BioResource Center Cell Bank [30], BAC clones used for the production of transgenic and knockout mice are not available to the public. We have constructed a BAC library of the B6N substrain and the RIKEN BioResource Center will complete both 5'- and 3'-end sequencings of B6N and register the sequence data with the DDBJ in collaboration with National Institute of Genetics under the Genome Information Upgrading Program of MEXT NBRP. As a result of this program, we will be able to provide scientists a BAC library of B6N enriched with end-sequencing information, which will enable genetic engineering with high accuracy using B6N ES cells and facilitate functional genomics studies.

Promoter collection

Recently, needs of knockout animal have increased, in particular, the ability to delete a specific gene in a spatio-temporal manner. For this purpose, it is important to collect and maintain genetic materials which can express an introduced gene in tissue have and developmental stage-specific manner.

We have constructed reporter vectors harboring human and mouse promoters which are known to regulate the expression of genes in stage- and tissue-specific manners. The constructed clones were analyzed with luciferase activity using 18 lines of cultured cells, and the results have been published on the web site of RIKEN BioResource Center. Among them, promoters which have a tissue-specific [human alpha 1-antitrypsin promoter (AAT)] or ubiquitous [cytomegalovirus early promoter (CMV)] activity of gene expression were chosen to construct an expression vector expressing a nuclear Cre recombinase [13] to generate Cre-transgenic mice (Cre-Zoo) in collaboration with the BRC Experimental Animal Division. The established transgenic animals have been deposited at the BRC Experimental Animal Division [34] and are available for access by researchers (The accession numbers in the Experimental Animal Division are as follows. AAT, RBRC02153, RBRC02155, RBRC02157; CMV, RBRC01910, RBRC01963).

Recombinant adenovirus

Many researchers have been using recombinant adenovirus with great success in the research fields of cancer therapy. Its numerous advantages have made recombinant adenoviral vectors the preferred gene delivery tool for mammalian cells. However, generating a re-

combinant adenovirus may be time-consuming. In order to overcome this time constraint, we provide recombinant ready-to-use adenoviruses [31]. Researchers may perform their own experiment using our genetic materials without reconstruction of the viral vectors. We have constructed and collected more than 500 recombinant adenoviruses and shuttle vectors to generate recombinant adenoviruses which can be used for the infection of individual mice as well as cultured mammalian cells [18].

Using Cre expressing recombinant adenoviruses [13], researchers can obtain cells which have only transient Cre expression because recombinant adenoviruses do not integrate into the genome of the host cells. Thus, a recombinant adenovirus is used for abrogating the expression of a target gene in ES cells or fibroblasts from floxed mice [8, 9, 24]. Cre- expressing recombinant adenovirus is also useful for removing the neo cassette, which is often used as a marker for the knockout allele of gene of interest, in ES cells to avoid the interference and genetic ambiguity arising from the use of the selected gene. It has been reported that removal of the floxed neo gene from a knockout allele is possible by infecting morulae at the 16-cell stage with recombinant Cre adenovirus to produce mosaic mice which transmit the desired allele without the neo cassette with high frequency to their offspring [12].

We also provide a rapid method for the purification of recombinant virus and the detection of contamination of replication competent adenoviruses [28, 32]. We also hold training courses in advanced techniques for the best handing of recombinant viruses and host bacteria.

Genetic Materials with Licensed Technology or Products

RIKEN BRC sets the Material Transfer Agreements (MTA) for each transfer to protect the intellectual property rights of the developers of genetic materials and to define the responsibility of users. We have opened a path to the academic use of genetic materials produced by research tools owned by commercial entities. RIKEN BRC has executed a license agreement with the Life Technologies Corporation (former Invitrogen IP Holdings, Inc.) to receive, maintain, replicate and distribute Gateway® Entry clones and Expression clones. The Promega Corporation has also agreed to the deposition of the recombinant derivatives of Promega's reporter vectors. Through the generosity of these biotechnology companies, RIKEN BRC is now able to distribute genetic materials to nonprofit organizations for not-for-profit academic research under this agreement. Thus, the core facility of the genetic materials at the NBRP is recognized as a reliable dissemination partner by these private entities.

How to Use the Genetic Materials

Users can access our genetic materials through the websites of RIKEN BRC (Fig. 4). Users must complete an MTA for Distribution form and an order form. Sometimes permission for use is required from the Depositor using an approval form. In addition, recombinant DNA experiments in Japan must be conducted with special attention to laboratory safety and environmental conservation. Therefore, all recipients of genetic resources must submit their experiment plans and protocols to their organization's Recombinant DNA Experiments Committees to obtain authorized approvals. RIKEN BRC starts to prepare the genetic materials upon receipt of the order documents from the users. We provide genetic materials for a minimal fee solely to reimburse the RIKEN BRC for a part of the preparation and handling costs of the requested genetic materials. This reimbursement fee is indispensable to continuing our activities of distribution. Recipients will also bear the shipping cost. The recipient of the genetic materials must expressly describe that "the genetic material was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan", in the Materials and Methods section of all publication.

Deposition of genetic materials at RIKEN BRC frees researchers from cumbersome preservation and distribution of materials to fellow researchers. Furthermore, deposition increases the chances of collaboration and citation in a research paper. You are welcome to deposit genetic resources at the Gene Engineering Division, RIKEN BRC.

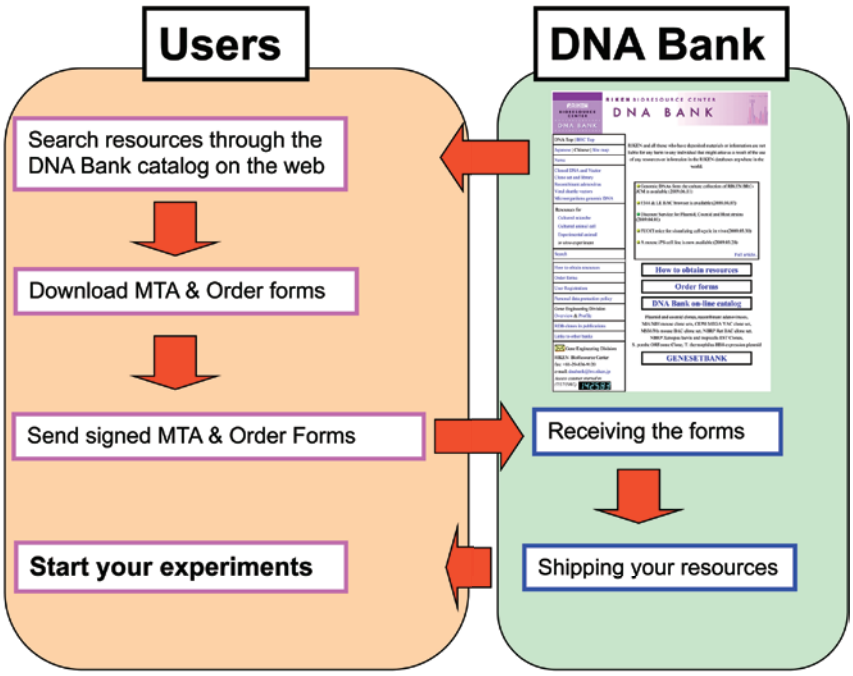

Fig. 4. Schematic flow diagram showing how to use our genetic materials. Users can easily access our genetic materials through the internet. Forms can be obtained at http://www.brc.riken.jp/lab/dna/en/furnish.html.

For the Best Experimental Results

Preservation

The RIKEN Gene Engineering Division performs authentication tests and determines the stability of all its genetic materials. It also develops methods for maintenance and preservation that are appropriate for the various biological materials, using appropriate technology. These records are retained and are useful as baseline information when in-storage maintenance checks are performed and for validation after preservation and/or restocking. A scheme for the periodic assessment of all preserved materials is in place for each item stored. The Division selects methods on the basis of recommendations from the depositors and previous experience. It documents all preservation procedures to ensure that they are reproducible and all key parameters are monitored.

The genetic materials are preserved, at the minimum, as duplicate collections, namely master stocks and distribution stocks. The labels include the batch date or number and the RIKEN DNA Bank accession number (RDB No.). To ensure a minimum number of transfers or generations from the original materials, when appropriate, the Division uses the distribution stock for supplying the genetic resource. All genetic resources are stored under environmental parameters that assure the stability of their properties. The RIKEN DNA Bank provides, upon request, all methods and procedures used in validation and the results of all validations.

Distribution of genetic resources

The following conditions apply to all items for distribution. First, the original developers maintain their priority access to DNA clones. Second, recipients must state that the DNA clones or their derivatives will not be used in human experiments. Third, DNA clones and their derivatives must be used for research purposes only and must not be given or sold to a third party. Fourth, distribution of some genetic resources are subjected to the written approval of the depositor or developer. Fifth, the recipient must acknowledge the original developer and note in all publications that clones were obtained from the RIKEN Gene Engineering Division. Finally,

Table 2. Relevant URLs

RIKEN BRC	http://www.brc.riken.jp/
Gene Engineering Division, RIKEN BRC	http://www.brc.riken.jp/lab/dna/en/
Experimental Animal Division, RIKEN BRC	http://www.brc.riken.jp/lab/animal/
Cell Engineering Division, RIKEN BRC	http://www.brc.riken.jp/lab/cell/
Japan Collection of Microorganisms (JCM, Japan), RIKEN BRC	http://www.jcm.riken.jp/
MEXT	http://www.mext.go.jp/
NBRP	http://www.nbrp.jp/index.jsp
Ciona intestinalis EST	http://www.brc.riken.jp/lab/dna/en/NBRPCiona_en.html
Xenopus EST	http://www.brc.riken.jp/lab/dna/en/xenopus_en.html
Human SEREX	http://www.brc.riken.jp/lab/dna/en/serex_ID.html
Human HLA	http://www.brc.riken.jp/lab/dna/en/GENESETBANK/HLA.html
Full CDS expression clones	http://www.brc.riken.jp/lab/dna/en/RDB5956_2en.html
Japanese macaque (Macaca fuscata) BAC	http://www.brc.riken.jp/lab/dna/en/NBRPmacaqueen.html
MSM/Ms BAC	http://www.brc.riken.jp/lab/dna/en/MSMBACen.html
Rat BAC	http://www.brc.riken.jp/lab/dna/en/NBRPraten.html
Promoter Collection	http://www.brc.riken.jp/lab/dna/en/promoteren.html
Recombinant Adenovirus	http://www.brc.riken.jp/lab/dna/en/adenovirus.html
NIBIO (JCRB) GeneBank	http://genebank.nibio.go.jp/gbank/index.html
JHSF	http://www.jhsf.or.jp/index_b.html
AFFRC GeneBank	http://www.dna.affrc.go.jp/misc/bank/indexJ.html
NITE Biological Resource Center	http://www.nbrc.nite.go.jp/e/index.html

the developer (depositor) and recipient shall uphold the terms and conditions of the MTA.

We pack and send requested genetic materials according to current postal and quarantine regulations. Invoices are normally dispatched with the materials unless otherwise instructed. We keep records of all requests for genetic materials, dates of shipment, and names and addresses of recipients. We also record all user queries and complaints and, when possible, acknowledge such communications by return of post, fax or e-mail. We investigate complaints as rapidly as possible and implement corrective action as necessary.

Education and training

Our division holds training programs for scientists and young students to teach the application of scientific technologies. Currently these programs focus on the technology of recombinant adenoviruses.

Homepage, catalogs, newsletters, and "E-mail news"

Materials are listed on our Homepage (http://www.brc.riken.jp/lab/dna/en/) or the Gene Catalog. We also send scientists Newsletters and the E-mail News every month.

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References

1. Abe, K., Noguchi, H., Tagawa, K., Yuzuriha, M., Toyoda, A., Kojima, T., Ezawa, K., Saitou, N., Hattori, M., Sakaki, Y., Moriwaki, K., and Shiroishi, T. 2004. Contribution of asian mouse subspecies *Mus musculus molossinus* to genomic constitution of strain C57BL/6J, as defined by BAC-end sequence-SNP analysis. *Genome Res*. 14: 2439–2447.
2. Akatsuka, Y., Goldberg, T.A., Kondo, E., Martin, E.G., Obata, Y., Morishima, Y., Takahashi, T., and Hansen, J.A. 2002. Efficient cloning and expression of HLA class I cDNA in human B-lymphoblastoid cell lines. *Tissue Antigens* 59: 502–511.
3. Akatsuka, Y., Kondo, E., Taji, H., Morishima, Y., Yazaki, M., Obata, Y., Kodera, Y., Riddell, S.R., and Takahashi, T. 2002. Targeted cloning of cytotoxic T cells specific for minor histocompatibility antigens restricted by HLA class I molecules of interest. *Transplantation* 74: 1773–1780.
4. Akatsuka, Y., Morishima, Y., Kuzushima, K., Kodera, Y., and Takahashi, T. 2007. Clinical relevance of a newly identified HLA-A24-restricted minor histocompatibility antigen epitope derived from BCL2A1, ACC-1, in patients

receiving HLA genotypically matched unrelated bone marrow transplant. *Cancer Sci*. 98: 1139–1146.
5. Akatsuka, Y., Nishida, T., Kondo, E., Miyazaki, M., Taji, H., Iida, H., Tsujimura, K., Yazaki, M., Naoe, T., Morishima, Y., Kodera, Y., Kuzushima, K., and Takahashi, T. 2003. Identification of a polymorphic gene, BCL2A1, encoding two novel hematopoietic lineage-specific minor histocompatibility antigens. *J. Exp. Med*. 197: 1489–1500.
6. Buehr, M., Meek, S., Blair, K., Yang, J., Ure, J., Silva, J., McLay, R., Hall, J., Ying, Q.L., and Smith, A. 2008. Capture of authentic embryonic stem cells from rat blastocysts. *Cell* 135: 1287–1298.
7. Cozzi, J., Fraichard, A., and Thiam, K. 2008. Use of genetically modified rat models for translational medicine. *Drug Discov. Today* 13: 488–494.
8. Dali-Youcef, N., Mataki, C., Coste, A., Messaddeq, N., Giroud, S., Blanc, S., Koehl, C., Champy, M.F., Chambon, P., Fajas, L., Metzger, D., Schoonjans, K., and Auwerx, J. 2007. Adipose tissue-specific inactivation of the retinoblastoma protein protects against diabesity because of increased energy expenditure. *Proc. Natl. Acad. Sci. U.S.A*. 104: 10703–10708.
9. Gao, Z., Sasaoka, T., Fujimori, T., Oya, T., Ishii, Y., Sabit, H., Kawaguchi, M., Kurotaki, Y., Naito, M., Wada, T., Ishizawa, S., Kobayashi, M., Nabeshima, Y., and Sasahara, M. 2005. Deletion of the PDGFR-beta gene affects key fibroblast functions important for wound healing. *J. Biol. Chem*. 280: 9375–9389.
10. Gill, T.J. 3rd, Smith, G.J., Wissler, R.W., and Kunz, H.W. 1989. The rat as an experimental animal. *Science* 245: 269–276.
11. Isa, T., Yamane, I., Hamai, M., and Inagaki, H. 2009. Japanese macaques as laboratory animals. *Exp. Anim*. 58: 451–457.
12. Kaartinen, V. and Nagy, A. 2001. Removal of the floxed *neo* gene from a conditional knockout allele by the adenoviral Cre recombinase *in vivo*. *Genesis* 31: 126–129.
13. Kanegae, Y., Lee, G., Sato, Y., Tanaka, M., Nakai, M., Sakaki, T., Sugano, S., and Saito, I. 1995. Efficient gene activation in mammalian cells by using recombinant adenovirus expressing site-specific Cre recombinase. *Nuc. Acids Res*. 23: 3816–3821.
14. Kargul, G.J., Dudekula, D.B., Qian, Y., Lim, M.K., Jaradat, S.A., Tanaka, T.S., Carter, M.G., and Ko, M.S. 2001. Verification and initial annotation of the NIA mouse 15K cDNA clone set. *Nat. Genet*. 28: 17–18.
15. Li, P., Tong, C., Mehrian-Shai, R., Jia, L., Wu, N., Yan, Y., Maxson, R.E., Schulze, E.N., Song, H., Hsieh, C.L., Pera, M.F., and Ying, Q.L. 2008. Germline competent embryonic stem cells derived from rat blastocysts. *Cell* 135: 1299–1310.
16. Liao, J., Cui, C., Chen, S., Ren, J., Chen, J., Gao, Y., Li, H., Jia, N., Cheng, L., Xiao, H., and Xiao, L. 2009. Generation of induced pluripotent stem cell lines from adult rat cells. *Cell Stem Cell* 4: 11–15.
17. Mekada, K., Abe, K., Murakami, A., Nakamura, S., Nakata, H., Moriwaki, K., Obata, Y., and Yoshiki, A. 2009. Genetic differences among C57BL/6 substrains. *Exp. Anim*. 58: 141–149.
18. Murata, T., Pan, J., Hirose, M., Inabe, K., Kujime, Y., Kurihara, C., Kusa, Y., Masuzaki, S., Nakade, K., Nakano, Y., Ohkubo, M., Yamasaki, T., Obata, Y., and Yokoyama, K.K. 2009. Introduction of recombinant virus bank in RIKEN Gene Engineering Division. *Gene Ther. Rev*. 1: 53–55.
19. Nagaso, H., Murata, T., Day, N., and Yokoyama, K.K. 2001. Simultaneous detection of RNA and protein by in situ hybridization and immunological staining. *J. Histochem. Cytochem*. 49: 1177–1182.
20. Nishida, T., Akatsuka, Y., Morishima, Y., Hamajima, N., Tsujimura, K., Kuzushima, K., Kodera, Y., and Takahashi, T. 2004. Clinical relevance of a newly identified HLA-A24-restricted minor histocompatibility antigen epitope derived from BCL2A1, ACC-1, in patients receiving HLA genotypically matched unrelated bone marrow transplant. *Br. J. Haematol*. 124: 629–635.
21. Obata, Y., Takahashi, T., Sakamoto, J., Tamaki, H., Tominaga, S., Hamajima, N., Chen, Y.T., and Old, L.J. 2000. SEREX analysis of gastric cancer antigens. *Cancer Chemother. Pharmacol*. 46: S37–42.
22. Obata, Y., Takahashi, T., Tamaki, H., Tominaga, S., Murai, H., Iwase, T., Iwata, H., Mizutani, M., Chen, Y.T., Old, L.J., and Miura, S. 1999. Identification of cancer antigens in breast cancer by the SEREX expression cloning method. *Breast Cancer* 6: 305–311.
23. Saar, K., Beck, A., Bihoreau, M.T., Birney, E., Brocklebank, D., Chen, Y., Cuppen, E., Demonchy, S., Dopazo, J., Flicek, P., Foglio, M., Fujiyama, A., Gut, I.G., Gauguier, D., Guigo, R., Guryev, V., Heinig, M., Hummel, O., Jahn, N., Klages, S., Kren, V., Kube, M., Kuhl, H., Kuramoto, T., Kuroki, Y., Lechner, D., Lee, Y.A., Lopez-Bigas, N., Lathrop, G.M., Mashimo, T., Medina, I., Mott, R., Patone, G., Perrier-Cornet, J.A., Platzer, M., Pravenec, M., Reinhardt, R., Sakaki, Y., Schilhabel, M., Schulz, H., Serikawa, T., Shikhagaie, M., Tatsumoto, S., Taudien, S., Toyoda, A., Voigt, B., Zelenika, D., Zimdahl, H., and Hubner, N. 2008. SNP and haplotype mapping for genetic analysis in the rat. *Nat. Genet*. 40: 560–566.
24. Sakaki-Yumoto, M., Kobayashi, C., Sato, A., Fujimura, S., Matsumoto, Y., Takasato, M., Kodama, T., Aburatani, H., Asashima, M., Yoshida, N., and Nishinakamura, R. 2006. The murine homolog of SALL4, a causative gene in Okihiro syndrome, is essential for embryonic stem cell proliferation, and cooperates with Sall1 in anorectal, heart, brain and kidney development. *Development* 133: 3005–3013.
25. Sasakura, Y., Inaba, K., Satoh, N., Kondo, M., and Akasaka, K. 2009. *Ciona intestinalis* and *Oxycomanthus japonicus*, representatives of marine invertebrates. *Exp. Anim*. 58: 459–469.
26. Satou, Y., Yamada, L., Mochizuki, Y., Takatori, N., Kawashima, T., Sasaki, A., Hamaguchi, M., Awazu, S., Yagi, K., Sasakura, Y., Nakayama, A., Ishikawa, H., Inaba, K., and Satoh, N. 2002. A cDNA resource from the basal chordate *Ciona intestinalis*. *Genesis* 33: 153–154.
27. Serikawa, T., Mashimo, T., Takizawa, A., Okajima, R., Maedomari, N., Kumafuji, K., Tagami, F., Neoda, Y., Otsuki,

M., Nakanishi, S., Yamasaki, K., Voigt, B., and Kuramoto, T. 2009. National BioResource Project-Rat and related activities. *Exp. Anim.* 58: 333–341.
28. Suzuki, E., Murata, T., Watanabe, S., Kujime, Y., Hirose, M., Pan, J., Yamasaki, T., Ugai, H., and Yokoyama, K.K. 2004. A simple method for the simultaneous detection of E1A and E1B in adenovirus stocks. *Oncol. Rep.* 11: 173–178.
29. Tanaka, T.S., Jaradat, S.A., Lim, M.K., Kargul, G.J., Wang, X., Grahovac, M.J., Pantano, S., Sano, Y., Piao, Y., Nagaraja, R., Doi, H., Wood, W.H. 3rd, Becker, K.G., and Ko, M.S. 2000. Genome-wide expression profiling of mid-gestation placenta and embryo using a 15,000 mouse developmental cDNA microarray. *Proc. Natl. Acad. Sci. U.S.A.* 97: 9127–9132.
30. Tanimoto, Y., Iijima, S., Hasegawa, Y., Suzuki, Y., Daitoku, Y., Mizuno, S., Ishige, T., Kudo, T., Takahashi, S., Kunita, S., Sugiyama, F., and Yagami, K. 2008. Embryonic stem cells derived from C57BL/6J and C57BL/6N mice. *Comp. Med.* 58: 347–352.
31. Ugai, H., Murata, T., Nagamura, Y., Ugawa, Y., Suzuki, E., Nakata, H., Kujime, Y., Inamoto, S., Hirose, M., Inabe, K., Terashima, M., Yamasaki, T., Liu, B., Nakade, K., Pan, J., Kimura, M., Saito, I., Hamada, H., Obata, Y., and Yokoyama, K.K. 2005. A database of recombinant viruses and recombinant viral vectors available from the RIKEN DNA bank. *J. Gene Med.* 7: 1148–1157
32. Ugai, H., Yamasaki, T., Hirose, M., Inabe, K., Kujime, Y., Terashima, M., Liu, B., Tang, H., Zhao, M., Murata, T., Kimura, M., Pan, J., Obata, Y., Hamada, H., and Yokoyama, K.K. 2005. Purification of infectious adenovirus in two hours by ultracentrifugation and tangential flow filtration. *Biochem. Biophys. Res. Commun.* 331: 1053–1060.
33. VanBuren, V., Piao, Y., Dudekula, D.B., Qian, Y., Carter, M.G., Martin, P.R., Stagg, C.A., Bassey, U.C., Aiba, K., Hamatani, T., Kargul, G.J., Luo, A.G., Kelso, J., Hide, W., and Ko, M.S. 2002. Assembly, verification, and initial annotation of the NIA mouse 7.4K cDNA clone set. *Genome Res.* 2: 1999–2003.
34. Yoshiki, A., Ike, F., Mekada, K., Kitaura, Y., Nakata, H., Hiraiwa, N., Mochida, K., Ijuin, M., Kadota, M., Murakami, A., Ogura, A., Abe, K., Moriwaki, K., and Obata, Y. 2009. The mouse resources at the RIKEN BioResource center. *Exp. Anim.* 58: 85–96.